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(54) **PROTEIN EXPRESSION FROM MULTIPLE NUCLEIC ACIDS**

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(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

The current invention reports a method for the recombinant production of a secreted heterologous immunoglobulin in a CHO cell comprising the following steps: i) providing a CHO cell, which is adapted to growth in suspension culture, adapted to growth in serum-free medium, mycoplasma free, and virus free, ii) providing a vector comprising a prokaryotic origin of replication, a first nucleic acid conferring resistance to a prokaryotic selection agent, a second nucleic acid encoding the heavy chain of said heterologous immunoglobulin, a third nucleic acid encoding the light chain of said heterologous immunoglobulin, a fourth nucleic acid conferring resistance to a eukaryotic selection agent, iii) transfecting said CHO cell, wherein said transfecting comprises a) transfecting said CHO cell with said vector comprising a fourth nucleic acid conferring resistance to a first eukaryotic selection agent, b) selecting a CHO cell by growth in cultivation medium containing said first eukaryotic selection agent, c) transfecting said selected CHO cell with said vector comprising a fourth nucleic acid conferring resistance to a second eukaryotic selection agent different to said first eukaryotic selection agent, d) selecting a CHO cell by selected growth in cultivation medium containing said first and said second eukaryotic selection agent, iv) cultivating said transfected CHO cell in a medium in the presence of said first and second eukaryotic selection agent, under conditions suitable for the expression of said second, and third nucleic acid, and v) recovering said secreted heterologous immunoglobulin from the cultivation medium.

11 Claims, 9 Drawing Sheets